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Free Radical Scavenging Property of *Bombax ceiba* Linn. Root

[1]V. Jain, [2]S.K. Verma, [1]S.S. Katewa, [3]S. Anandjiwala and [3]B. Singh

[1]Laboratory of Ethnobotany and Agrostology, Department of Botany, University College of Science, Mohan Lal Sukhadia University, Udaipur, Rajasthan, India

[2]Indigenous Drug Research Center, Department of Medicine, RNT Medical College, Udaipur-313001, Rajasthan, India

[3]Department of Phytochemistry and Pharmacognosy, B.V. Patel Pharmaceutical Research and Development Centre, Ahmedabad, Gujarat, India

Corresponding Author: V. Jain, Laboratory of Ethnobotany and Agrostology, Department of Botany, University College of Science, Mohan Lal Sukhadia University, Udaipur, Rajasthan, India Tel: 91-294-2484809

ABSTRACT

Silk cotton tree (*Bombax ceiba* Linn.) is a well known ethnomedicinal plant. Root of this plant was investigated for its antioxidant potential for the first time. Assessment of antioxidant activity was done using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and Reducing power assay. Preliminary phytochemical screening of the roots showed presence of phenolics, tannins, flavonoids, steroids, saponins and cardiac glycosides. Methanolic extract of the roots showed high amount of phenolics (30.95% w/w) and tannins (15.45% w/w) and a very good DPPH radical scavenging activity (EC_{50} of 15.07 μg) in a dose dependent manner. The extract showed dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) with a maximum absorbance of 1.11 at a concentration of 500 μg of the extract. Acute study in healthy human volunteers showed a significant ($p<0.05$) rise in total antioxidant status at the end of 4 h after administration of 3 g root powder. This strong *in vitro* and *in vivo* antioxidant potential of *B. ceiba* dry root powder validates its uses in diabetes mellitus and heart disease as described in the traditional medicine.

Key words: Semal, silk cotton tree, antioxidant, sesquiterpenoids, DPPH, reducing power assay

INTRODUCTION

Excessive free radical production and lipid peroxidation has been shown as significant contributor to the process of atherosclerosis, ischemic heart disease, diabetes, carcinogenesis, neurodegenerative disorder, rheumatic disorders, aging etc. in humans (Tiwari, 2001, 2004). Various phytochemicals present in plants help in providing protection against cancer, cardiovascular diseases, dementia, cataract, macular degeneration, ageing and various other disorders associated with increased oxidative stress. These phytochemicals act as antioxidants which intercept free radicals and protect the cells from the oxidative damage (Nuttall *et al*., 1999).

Bombax ceiba Linn. (syn. *Bombax malabaricum* DC. *Salmalia malabarica* (DC.) Schott and Endl]; a large, deciduous tree, commonly known as Silk Cotton Tree, Indian Red Kapok tree, Semal, etc. is a member of family Bombacaceae. It is found throughout India and other parts of tropical and sub-tropical Asia, Australia and Africa (The Wealth of India, 2004). The plant is quite popular among the tribal communities for the treatment of various diseases of both human and animals and almost every part of the plant is employed as a medicine. Young roots of the plant have been

reported to be useful in diarrhoea, dysentery, urinary troubles, gynaecological problems, bladder disorders, heart diseases, debility, diabetes and impotence (Katewa and Jain, 2006; Jain *et al.*, 2009). Besides having immense medicinal potential, the plant has also been used for commercial and industrial purposes (The Wealth of India, 2004).

Recently, the plant has undergone extensive scientific scrutiny and research worldwide has shown that the flowers, leaves and stem of *B. ceiba* possess strong anti-inflammatory, antibacterial, antiviral, analgesic, oxytocic (Gupta *et al.*, 2004), antioxidant (Vieira *et al.*, 2009), hypotensive, hypoglycemic (Saleem *et al.*, 1999) antiangiogenic (You *et al.*, 2003) and hepatoprotective (Ravi *et al.*, 2010) activities. However, the studies on its root are limited. Lately the root has demonstrated fibrinolysis enhancing (Verma *et al.*, 2006) and antihyperglycemic (Verma *et al.*, 2008) properties in human volunteers. To establish the validity of the traditional phyto-therapeutic claims of the root of plant, the present work is the first attempt to investigate the antioxidant potential of *B. ceiba* root powder in two *in vitro* models and one *in vivo* study in healthy volunteers.

MATERIALS AND METHODS

In vitro experiments were conducted during July 2008 to April 2009 at B.V. Patel Pharmaceutical Education, Research and Development Centre, Ahmedabad and Department of Botany, M.L. Sukhadia University, Udaipur. *In vivo* study was done at Indigenous Drug Research Centre, Department of Medicine, RNT Medical College, Udaipur, Rajasthan, India during October 2009 to January 2010.

Collection and preparation of plant material: Young roots of *B. ceiba* were collected from the forest area situated near Udaipur district, Rajasthan, India. Plant sample was identified and a voucher specimen (No. EA-202) was deposited in the Laboratory of Ethnobotany and Agrostology, Department of Botany, M.L. Sukhadia University, Udaipur for future reference. Roots were cut in small pieces, air-dried in shade at an ambient temperature and filled in airtight glass containers. They were powdered to 40 meshes as and when required.

Preparation of methanolic extract: Fifty gram powder of *B. ceiba* roots was extracted with methanol (4×500 mL) under reflux. The extract was filtered, pooled and solvent was removed under reduced pressure.

Preliminary phytochemical evaluation: Five-hundred milligram of the dried methanolic extract was reconstituted in 10 mL of methanol and used for preliminary phytochemical testing for the presence of different chemical groups of compounds such as carbohydrates, amino acids, saponins, phenols, tannins, flavonoids, terpenoids, cardiac glycosides and steroids (Edeoga *et al.*, 2005; Mace-Gorbach, 1963; Anandjiwala *et al.*, 2007).

Estimation of total phenolics: The total phenolic content of the extract was estimated as described by Anandjiwala *et al.* (2007) and Singleton and Rossi (1965). It was expressed as % gallic acid.

Estimation of total tannins: Total tannin content was estimated by the method as described by AOAC (William, 1960).

Preparation of stock solution for assessment of *in vitro* free radical scavenging activity: Dried methanolic extract (100 mg) was dissolved in 100 mL of methanol to make a stock solution of 1 mg mL^{-1}. Aliquots from this stock solution were further diluted with methanol as per the concentrations required. Free radical scavenging activity of the methanolic extract was tested in two *in vitro* models as follows:

DPPH radical scavenging activity: Antiradical activity was measured by a decrease in absorbance at 516 nm of a methanolic solution of colored DPPH brought about by the sample (Anandjiwala *et al*., 2007; Vani *et al*., 1997). A stock solution of DPPH (1.3 mg mL^{-1} in methanol) was prepared such that 75 μL of it in 3 mL methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract at different concentrations was noted after 15 min. EC_{50} was calculated from % inhibition. A blank reading was obtained using methanol instead of the extract. Pyrogallol was used as positive control.

Suitably diluted stock solution of the methanolic extract were applied on precoated TLC plates with silica gel 60 F_{254} using Camag Linomat V automatic sample spotter and developed in appropriate solvent system (n- Butanol : Acetic acid : Water : Ethyl acetate : Methanol :: 5: 1: 4: 2 : 4). Then the air dried TLC plate was sprayed with 0.2 % DPPH in methanol. Bleaching of DPPH by the resolved bands was observed for ten min and the details were recorded.

Reducing power assay: The reducing ability of the methanolic extract was measured by the transformation of Fe^{3+} to Fe^{2+} in the presence of the extract at 700 nm (Oyaizu, 1986). Increased absorbance of the reaction mixture indicates increased reducing power. Different concentrations of extracts in one ml of water were mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. TCA (2.5 mL) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Then 2.5 mL of the upper layer solution was mixed with 2.5 mL distilled water and 0.5 mL of $FeCl_3$ solution and the absorbance was measured at 700 nm. Gallic acid and tannic acid were used as positive controls.

***In vivo* antioxidant study:** The study was approved by the institutional ethical committee. Ten, middle aged (50-60 years), male healthy volunteers were selected for the acute study. They were non obese, non smokers and stable in their dietary and exercise habits. They were subjected to relevant investigations to exclude underlying heart, kidney, liver, endocrine and metabolic diseases. They were administered 3 g of *B. ceiba* root powder, filled in gelatin capsules. Blood samples were collected in fasting state, both before and after 4 h of administration of the root powder, for estimation of Total antioxidant status (Miller *et al*., 1993) using the standard kit supplied by Randox, UK.

Statistical analysis: The results of *in vitro* study are given as Mean±Standard Deviation (SD) obtained from three independent experiments. The results of acute study were expressed as Mean±Standard Error (SE) and analyzed with Student's t-test for paired data and a 'p' value less than 0.05 was considered as significant difference in the analysis.

RESULTS AND DISCUSSION

Preliminary phytochemical testing of roots of *B. ceiba* showed the presence of steroids, saponins, flavanoids, cardiac glycosides and high amount of tannins and phenolics (Table 1). Subsequent

Table 1: Preliminary phytochemical screening of *Bombax ceiba* root powder

Chemical group	Observation
Carbohydrates	+++
Amino acid	+++
Phenol	+++
Tannin	+++
Flavanoids	++
Saponins	++
Cardiac glycosides	++
Steroids	++
Terpenoids	++

+++ Abundant, ++ Average

Table 2: Total phenolic and total tannin content in root of *Bombax ceiba*

Plant sample	Total phenolic content* (% w/w)	Total tannins* (% w/w)
Crude root powder	4.86±0.06	1.72±0.08
Methanolic extract	30.95±1.39	15.45±1.17

*Values are expressed as Mean±SD (n = 3)

Table 3: Antiradical activity of methanolic extract of *Bombax ceiba* root observed with DPPH

Sample	Concentration (µg)	%Inhibition*	EC_{50} (µg)
Methanolic extract	5	17.32±1.81	5.07
	10	29.42±1.20	
	20	57.51±0.32	
	30	71.12±0.77	
	40	87.85±1.26	
	50	96.81±0.32	
Pyrogallol			4.85

*Mean±SD (n = 3)

quantification of total phenolic content was found to be 30.95% w/w in the methanolic extract (4.86% of powdered drug) calculated as gallic acid. Total tannin content was 15.45% w/w in the methanolic extract (Table 2).

Methanolic extract showed a concentration dependent DPPH radical scavenging activity by bleaching it with an EC_{50} value of 15.07 µg which was quite comparable to that of the positive control pyrogallol (Table 3). TLC plate applied with the methanolic extract, when sprayed with 0.2% DPPH in methanol; showed a streak of discoloration from the application point to the solvent front (R_f 0.10 to 0.96) showing the presence of compounds having antiradical activity (Fig. 1).

The extract also showed dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) in reducing power assay; showing a maximum absorbance of 1.11 at a concentration of 500 µg of the methanolic extract comparable to that of Gallic and tannic acid which were used as positive control and gave maximum absorbance at a concentration of 50 µg (Table 4).

In acute study in healthy volunteers, serum total antioxidant status was found to be significantly ($p<0.05$) increased by 44% after administration of 3 g *B. ceiba* root powder in a single dose (Fig. 2).

Antioxidant compounds in food play important roles in disease prevention and health promotion. The screening of plant extracts and natural products for antioxidant and antimicrobial activity has revealed the potential of higher plants as a source of new agents to serve the processing

Table 4: Reducing power assay of methanolic extract of *Bombax ceiba* root

Sample	Concentration (µg)	Absorbance*
Methanolic extract	10	0.027±1.81
	50	0.172±2.20
	100	0.370±0.32
	200	0.554±0.46
	300	0.710±0.77
	500	1.111±2.26
Gallic acid	5	0.088±0.008
	10	0.183±0.001
	20	0.523±0.031
	50	1.218±0.015
Tannic acid	5	0.146±0.019
	10	0.306±0.008
	20	0.710±0.010
	50	1.482±0.034

*Mean±SD (n = 3)

Fig. 1: TLC profile of methanolic extract of *Bombax ceiba* root after spraying with 0.2% methanolic DPPH

of natural products (Mokbel and Suganuma, 2006). Use of natural antioxidants, as food additives for inactivating free radicals receives a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products and their appreciation by consumers are very favorable.

Bombax ceiba Linn. is a phyto-pharmaceutical employed in traditional systems of medicine for treatment of various oxidative stress mediated diseases. Various parts of *B. ceiba* have shown to possess strong antioxidant potential. Mangiferin; isolated from its leaves has shown DPPH radical scavenging activity with an IC_{50} of 5.8±0.96 µg mL^{-1} (Dar *et al*., 2005). Gum of this plant has also shown good antioxidant potential in DPPH, FRAP and ABTS radical scavenging assay (Surveswaran *et al*., 2007). Recently, Vieira *et al*. (2009) has reported antioxidant activity of methanolic extract of its flowers against DPPH, hydroxyl free radicals and lipid peroxidation.

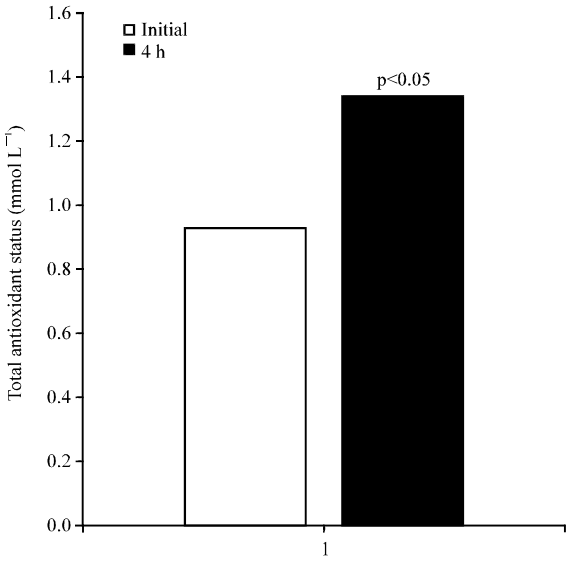

Fig. 2: Acute effect of 3 g *Bombax ceiba* root powder administration on total antioxidant status in healthy volunteers (n = 10)

Root of the plant is though scientifically less evaluated, yet has many medicinal activities such as stimulant, emetic, astringent, antidiarrhoeal, antidysenteric, aphrodisiac, demulcent, hemostatic, tonic (Gupta *et al*., 2004) and has been used in ethnomedicine to treat spermatorrhoea, leucorrhoea, gonorrhoea, bed wetting, impotency, diabetes, liver complaints, boils, burns, urine complaints, menorrhagia, syphilis and common cold (Jain *et al*., 2009). Recent scientific investigations of its roots have proved its anti-inflammatory, hepatoprotective (Lin *et al*., 1992), anti *H. pylori* (Wang and Huang, 2005), fibrinolysis enhancing (Verma *et al*., 2006) and anti-hyperglycemic (Verma *et al*., 2008) properties. However, this study is first attempt to evaluate the antioxidant property of roots of *B. ceiba*.

Synthetic antioxidants have not proved very useful as compared to plant derived natural antioxidants because the advantage of using natural antioxidants is that they might provide more useful flavonoids and other antioxidant compounds not present in standard oral synthetic antioxidants (Vievkanathan *et al*., 2003). Looking to all this, the present investigation is an important step in developing new plant based antioxidant therapeutic agent.

Hydrogen donating ability is an index of the primary antioxidants. DPPH is commonly used as a tool to evaluate the free radical scavenging activity of new compounds. It is a nitrogen centered stable free radical which is reduced when it receives an electron or hydrogen atom and (Mensor *et al*., 2001; Muchuweti *et al*., 2007). Root extract of *B. ceiba* showed a good dose dependent DPPH radical scavenging activity with an EC_{50} value of 15.07 μg (Table 3). This simple test model may be helpful in identifying antioxidant molecules present in the roots of *B. ceiba*, useful for development of anticancer, antiathersclerotic, antidiabetic therapeutics and neuroprotective agents.

Reducing power assay is another convenient and rapid screening method for measuring the antioxidant potential (Oyaizu, 1986; Chanda *et al*., 2011). Reducing power of a compound is related to electron transfer ability of that compound and therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). The

reduction ability of *B. ceiba* root was found to increase with rising concentrations of methanolic extract and 500 μg of the extract was shown to have maximum reducing power (Table 4).

Many plants exhibit efficient antioxidant properties owing to their phenolic constituents (Larson, 1988). Chemical analysis of *B. ceiba* root has revealed that it contains lupeol, β-sitosterol besides phenolic compounds, sesquiterpenes and napthoquinones (Seshadri *et al*., 1971; Puckhaber and Stipanovic, 2001; Reddy *et al*., 2003). Recently some new sesquiterpenoids named as Bombamalones A-D, Bombamaloside, Lacinilene, Bombaxquinone have also been isolated from the roots by Zhang *et al*. (2007).

Phenolic compounds easily donate hydroxyl hydrogen due to resonance stabilization (Fessenden and Fessenden, 1994). Roots of *B. ceiba* have shown presence of high amounts of total phenolic content and tannins. Combining this fact with the obtained results we could suggest that as the amount of phenolic compounds increases, reducing power increases as well.

In support of results obtained through *in vitro* analysis, a significant rise in serum total antioxidant status of human volunteers after administration of a single dose of 3 g root powder was an important observation obtained in the present study. In a similar study, an acute rise in plasma antioxidant status of healthy volunteers has been shown after consumption of different fruit juices (Ko *et al*., 2005) which substantiate role of plants as enhancers of antioxidant status in man. Hence, this potent antioxidant property further establishes the efficacy of root of *B. ceiba* in providing protection against oxidative stress mediated diseases such as diabetes and heart disease for which it is well recommended in the traditional systems of medicine.

It can therefore, be concluded that the root of Silk Cotton Tree possesses strong antioxidant potential and this may be due to the presence of phenolic compounds, sesquiterpenoids and napthoquinones. The antioxidant activity observed *in vitro* and in human volunteers, validates its uses as described in traditional medicine. Further studies should be aimed towards isolating the active principle of the plant having this antioxidant potential and evaluating it in a large number of subjects, so that it can be utilized commercially as a plant based therapeutic agent.

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